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The development of a novel urea sensor using polypyrrole

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ABSTRACT

The urease enzyme (Urs) was successfully incorporated into a polypyrrole film (PPy) in one simple electropolymerisation step. The films were formed using different dopant anions. The polypyrrole-urease-chloride film (PPy-Urs-Cl) was deposited from a simple chloride dopant, and the polypyrrole-urease-sulfonated-β-cyclodextrin (PPy-Urs-SCD) was formed using a sulfonated-β-cyclodextrin (SCD) dopant. The presence of the Urs within the polymer film was evident from the fibrous morphology, observed in the SEM micrographs, and the presence of nickel, arising from the active site of the urease enzyme. The sensing ability of the films and their enzyme-free counterparts (i.e., PPy-Cl and PPy-SCD) towards urea was investigated. The dopant anion plays an important role in the sensitivity of the polymer films towards urea. The PPy-Urs-SCD film has a superior sensitivity of 5.79 μC μM^{-1} compared to 0.76 μC μM^{-1} for the PPy-Urs-Cl polymer film. Furthermore, the negative groups on the SCD eliminate interference from common interfering compounds, such as ascorbic acid.

1. Introduction

Urea is an important molecule in both the agricultural and medical industries and it is often monitored in the blood as it can act as an indicator of renal disease in the human body. Excess nitrogen in the form of urea is dissolved in the blood and then excreted by the kidneys as a component of urine. In addition, a small amount of urea is also excreted *via* sweat/perspiration, along with salts and water. If this excess nitrogen is not excreted, ammonia can build up in the body to high levels which leads to cell toxicity and eventually to death [1]. The normal blood levels of urea range from 2.5 to 7.5 mM, depending on the build and relative health of the body [1]. Above 7.5 mM, the patient is said to be suffering from renal deficiency, and the kidneys fail to excrete the excess nitrogen successfully. Hence, it is very important to monitor the level of urea to determine the health of the kidneys in the human body [2,3].

The simplest method of monitoring the urea concentration is to immobilise the urease enzyme (Urs) onto an electrode. This has been widely investigated throughout the literature and proves to be the most promising approach [2]. The urease enzyme can be immobilised onto an electrode by covalent binding to a conducting polymer film, or by entrapment during the electrodeposition of the polymer film onto the electrode. A wide range of conducting polymers has been used for the entrapment of urease, including polypyrrole (PPy), polyaniline (PAni) and polythiophene, and their derivatives [4].

The immobilised urease electrode was first proposed in the 1970s by Guilbault and Montalvo [5], who monitored the formation of ammonium ions from urea, catalysed by the urease enzyme as described in Equation 1.

$$NH_2CONH_2 + 3H_2O \xrightarrow{Urease} 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

Since then, the entrapment of urease within a polymer matrix has gained a lot of attention, with polypyrrole and polyaniline being the most common choice of conducting polymers [6–10]. An electroactive polyaniline film has been used for urea detection whereby the urease enzyme was entrapped within the electroactive polymer during electrodeposition [9,10]. An electroactive polypyrrole with a polyion complex has been utilised as a composite film for the detection of urea, where the polyanion included PAA (polyacrylate) and PSS (polystyrene sulfonate) with PLL (polylysine) as the polycation. The urease enzyme was entrapped within the polyion composite film and a stable, rapid response signal for urea was achieved [7,8]. Additionally, Adeloju *et al.* [11,12] have developed a polypyrrole-urease (PPy-Urs) film that detects urea using flow injection analysis, a concept that was also utilised by Walcerz and co-workers with promising results [13]. In more recent years, urea has been detected by ion selective optical sensors that detect the ammonium ions generated from urea via the urease enzyme [14] and by ion selective

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field effect transistors (ISFETs) [15–17]. Co-polymers have also been employed. Rajesh *et al.* [18–20] synthesised a polypyrrole based co-polymer for the amperometric detection of urea with a short response time and good electrode stability.

This paper is focused on the development of a novel urea sensor formed by the entrapment of the urease enzyme within a polypyrrole matrix. This has been carried out previously [1,21], however, poor detection limits were obtained. To further enhance the detection of urea in solution, while repelling common interferants, such as uric acid and ascorbic acid, an anionic cyclodextrin was incorporated into the polypyrrole matrix together with the urease enzyme.

2. Experimental

2.1. Chemicals

The chemicals used throughout this study were purchased from Sigma-Aldrich or its subsidiary company Fluka. The urease enzyme chosen was the Jack Bean Urease from the *Canavalia ensiformis* plant as it contains two nickel atoms in its active site [2], which would be easy to detect using techniques such as SEM and EDX. The urease enzyme was dissolved in the electropolymerisation solution to give a concentration of 4000 mg dm^{-3}. All chemicals were used as supplied expect for pyrrole which was vacuum-distilled and stored in the dark at -20 °C prior to use. All other solutions were made from a stock solution of pH 7.0, 0.05 M phosphate buffer, which was initially prepared using distilled water. This concentration of phosphate buffer was chosen as higher concentrations are known to interfere with the biocatalytic activity of urease, whereas lower concentrations have insufficient conductivity [9]. All of the solutions were freshly prepared before each experiment.

2.2. Instrumentation

Potentiostatic and cyclic voltammetry experiments were carried out using a Solartron Potentiostat Model 1287. All measurements were performed at room temperature (approximately 25 °C) in a standard three-electrode cell with a platinum (Pt) working electrode, a high surface area platinum wire counter electrode and a SCE reference electrode. The Pt electrodes (4 mm in diameter) were encased in a larger Teflon® sheath and set in place using a non-conducting epoxy resin. The electrical contact was made with a copper wire attached using a highly conducting silver-loaded resin. The working electrodes were polished to a smooth surface, mirror finish, using 30, 15, 6 and 1 μm diamond suspensions on microcloth (Buehler), sonicated in distilled water and then in ethanol to remove any polishing residues, and finally rinsed with distilled water and dried.

2.3. Fabrication of the Urs immobilised into polypyrrole (PPy) films

The Urs was immobilised into the polypyrrole (PPy) films in a single-step by physical entrapment of the enzyme into the conducting polymer during electrodeposition. The films were electrochemically prepared onto the platinum working electrode at a fixed potential of 0.70 V vs. SCE from an aqueous solution containing pyrrole monomer (0.50 M), Urs (4000 mg L^{-1}) and NaCl (0.10 M) for the PPy-Urs-Cl films and from a solution containing pyrrole monomer (0.50 M), Urs (4000 mg L^{-1}) and sulfonated-β-cyclodextrin (0.02 M) for the PPy-Urs-SCD films. The PPy-Cl and PPy-SCD films were prepared in the absence of the urease enzyme for comparative purposes. Although it would be expected that the activity of the Urs would decrease upon its immersion in the conducting electrolyte [22], no significant decrease of the enzyme activity was observed. The polymer films were deposited until a fixed charge of 0.10 C cm^{-2} was achieved. The thickness of the films obtained was approximated as 3.55 μm, which was theoretically calculated using the charge thickness ratio derived by Diaz *et al.* [23] for a simple chloride dopant. In this analysis it is assumed that 1.0 C cm^{-2} of charge passed is equivalent to 2.5 μm of polymer film. It is important to mention that the theoretical values of thickness obtained for the PPy-Urs-Cl, PPy-SCD and PPy-Urs-SCD films are only an approximation, as the films doped with the large anionic groups may not have the same charge to polymer thickness ratio as the PPy-Cl films [24,25]. All of these films were characterised using SEM and EDX analysis and then investigated as suitable sensors for the detection of urea.

3. Results and Discussion

3.1. Formation of PPy-Urs using a potentiostatic mode

The urease enzyme was incorporated into the polymer film by physical entrapment during the deposition of the polymer at a fixed potential of 0.70 V vs. SCE. The PPy-Urs-Cl film was deposited from a 0.10 M NaCl solution containing 4000 mg L^{-1} Urs and 0.50 M pyrrole, while the enzyme-free, PPy-Cl, was deposited from a pyrrole solution containing 0.10 M NaCl. The sulfonated-β-cyclodextrin (SCD), which is a polyanion as shown in Fig. 1, has a high conductivity and a 0.02 M SCD solution with 4000 mg L^{-1} Urs and pyrrole was used to deposit the PPy-Urs-SCD film at 0.70 V vs. SCE. The corresponding film in the absence of urease was also formed.

The current-time plots recorded during the formation of the PPy-Urs-Cl, PPy-Cl, PPy-Urs-SCD and PPy-SCD films are shown in Fig. 2. It is clear that the current-time plots for the chloride-containing films differ significantly from the data recorded for the SCD-containing films. Initially, there is a rapid decrease in the current, which arises from the charging of the double layer. This is

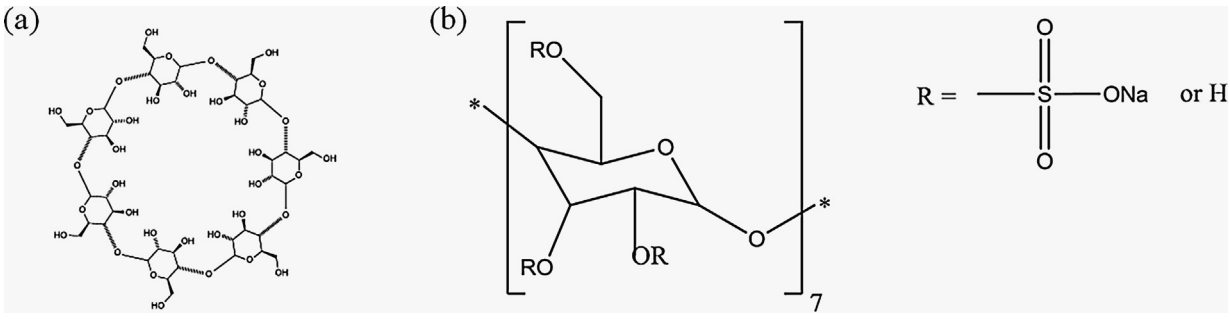

Fig. 1. (a) The structure of beta cyclodextrin in the absence of sulfonated molecules and (b) the formula and structure of sulfonated beta cyclodextrin (SCD).